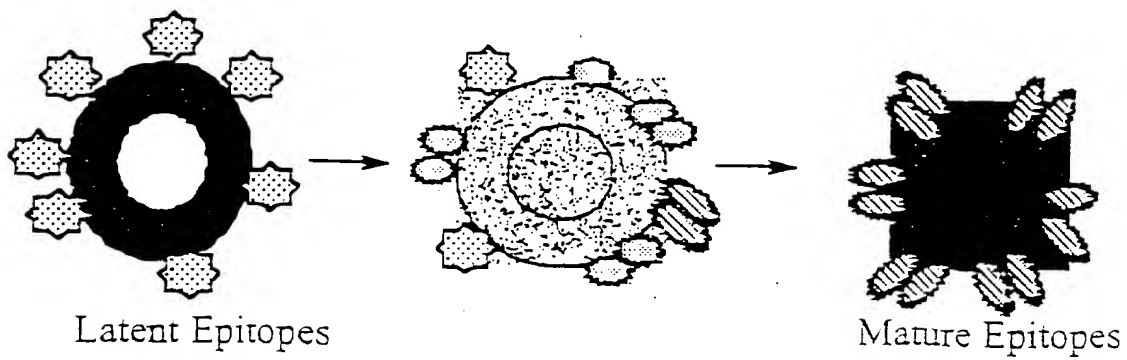


Scheme 1. Conformational search and chemical conversion during rHBsAg maturation

A. Schematic Representation for Epitope Evolution



B. Chemical Conversion - Interchain Disulfide Bond Cross-linking

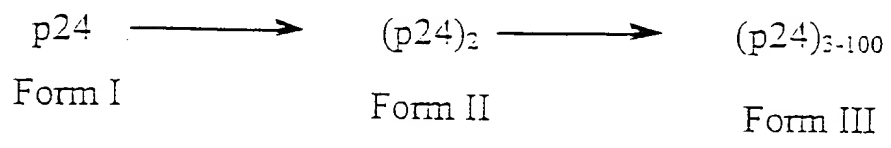
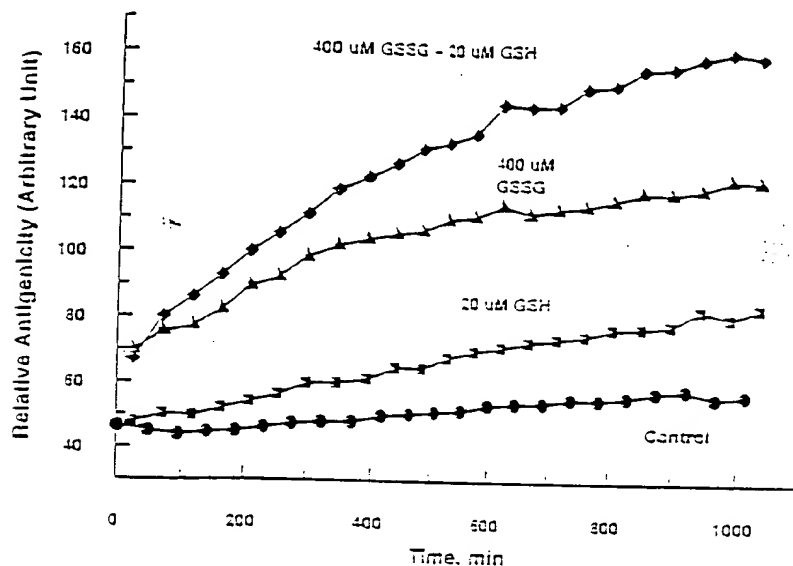
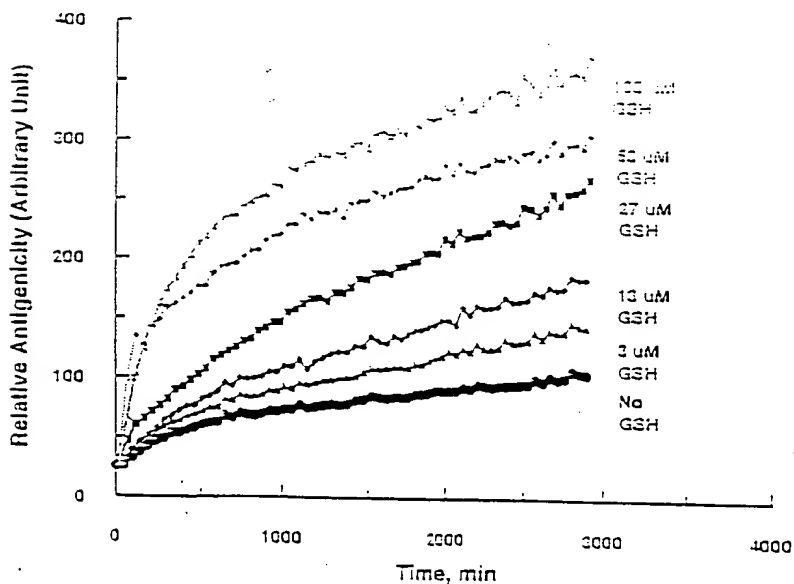


FIG 2

Glutathione-mediated maturation of rHBsAg at 37 °C. Top: Synergistic effects of GSH and GSSG; Bottom: Better conformation of HBsAg can be achieved by higher concentration of GSH.



GSH-Catalyzed Unscrambling of Disulfide Bond(s) During HBsAg



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A1.2	1	10	20	30	40	50	60																																																						
	D	I	V	L	T	Q	S	P	A	I	M	S	A	S	P	Q	E	K	V	T	M	T	C	S	A	S	S	S	V	S	Y	M	Y	-	M	Y	Q	Q	K	P	Q	S	S	P	R	L	L	I	F	10	T	S	M	L	F	S		G	V	P	V

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FIG 4

Flow Chart

In Vitro Relative Potency Assay

DAY 1

Pre-treat standard and samples - Section III.A.

Dispense 200 mcL of each treated standard or sample dilution into wells↓
Dispense 200 mcL of the Diluent Control and the Kit Controls into replicate wells↓
Add 50 mcL of conjugate to wells↓
Tap tray gently to mix↓
Add one bead to each test well↓
Incubate trays for 12-20 hours at 20-28°C

DAY 2

↓
Wash beads 1 cycle with 14 ± 3 mL distilled water↓
Transfer beads to tubes↓
Add 300 mcL of OPD substrate solution↓
Incubate 30 to 35 minutes at 20-28°C in the dark↓
Add 1 mL of 1N Sulfuric acid to each tube↓
Read tubes in Quantumatic™ within 2 hours

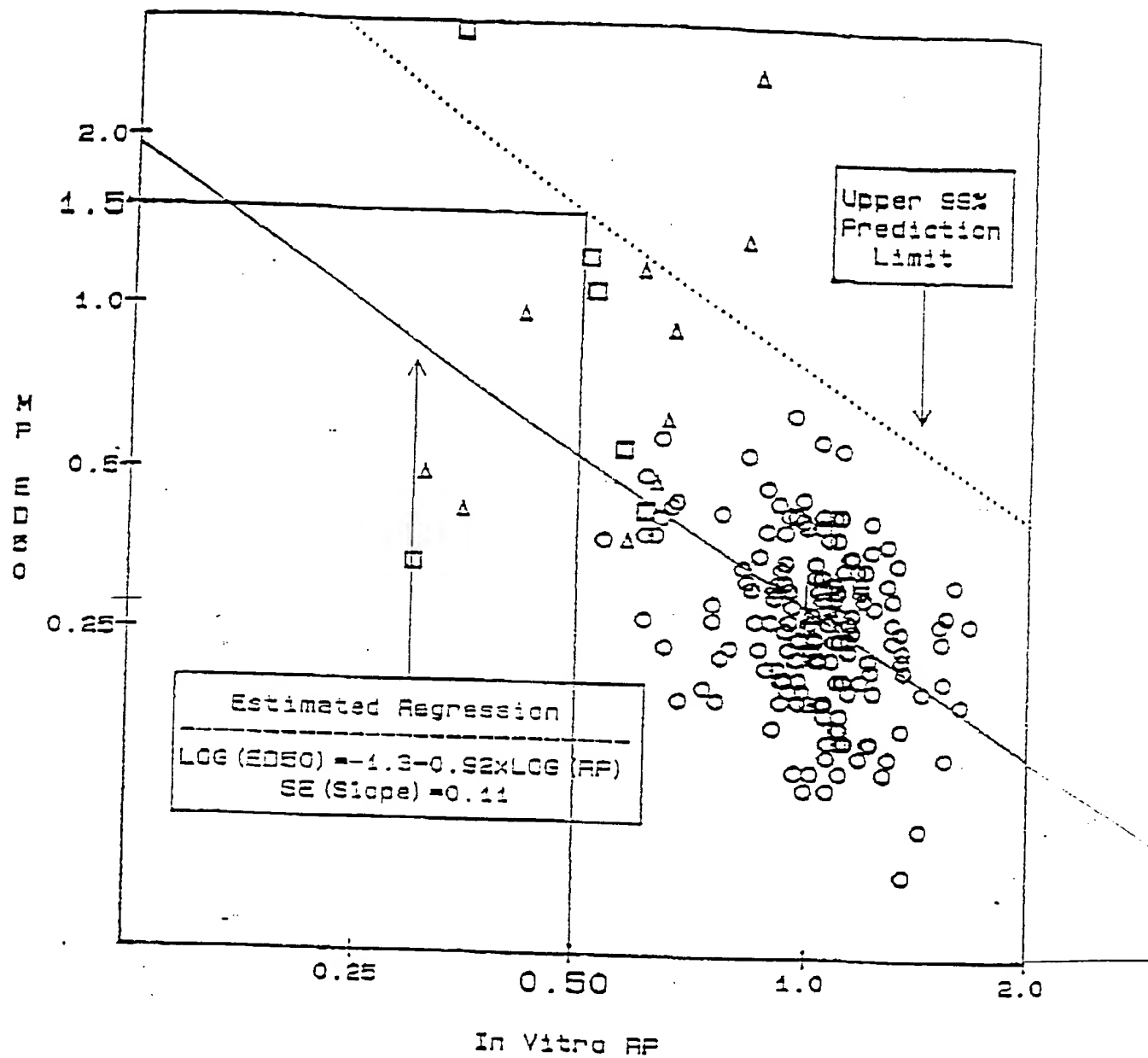
- Read Kit Controls blanked on substrate.

- Read Standards and test blanked on sample diluent.

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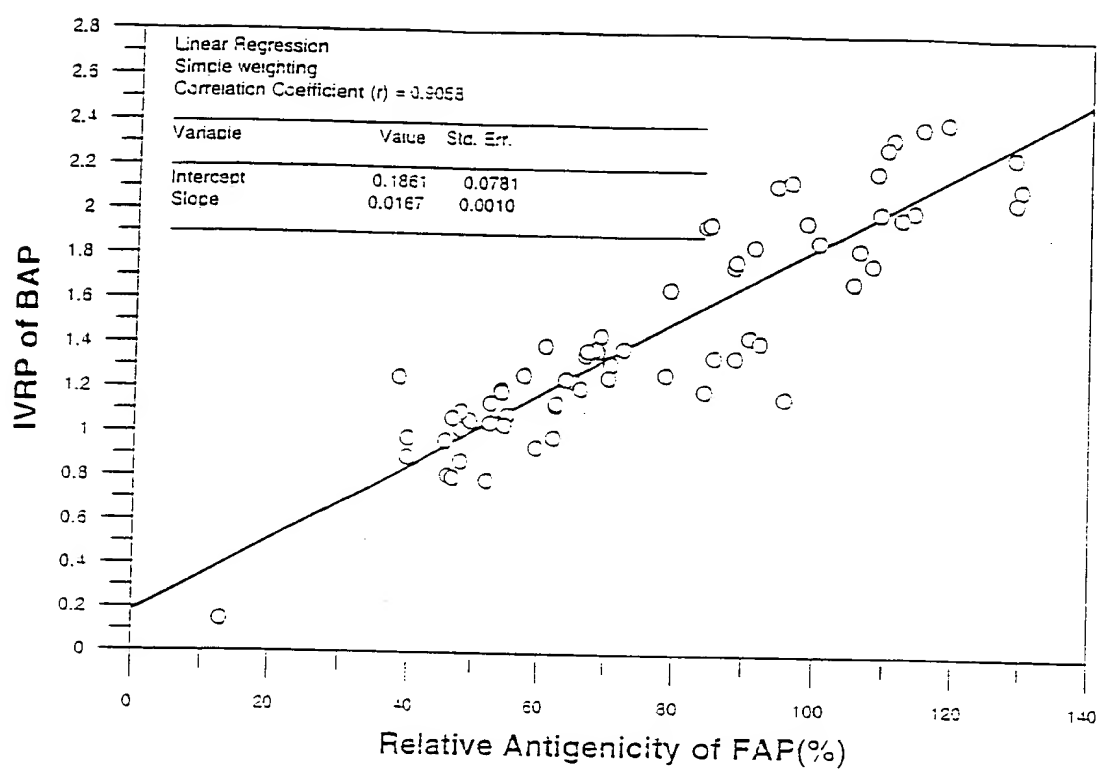
FIG 5

In Vitro Relative Potency Specification
Corresponding to Mouse Potency Specification



[illegible]

FIG 7



Linear relationship between IVRP of BAP vs. relative antigenicity of FAP (%) by EIAccre.